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(54) Title: PROTEIN KINASE C INHIBITORS (57) Abstract The present invention discloses compounds that are highly isozyme selective, protein kinase C beta-1 and beta-2 isozyme inhibitors. These compounds are 3,4-di-[3'-(1'-substituted)-indolyl]-1H-pyrrol-2,5-diones, which can be more commonly described as 3,4-bis(3'-indolyl) maleimides. Also, as isozyme selective inhibitors of the beta-1 and beta-2 isozymes, the compounds are therapeutically useful in treating conditions associated with diabetes mellitus and its complications, as well as other disease states associated with an elevation of these isozymes.		

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PROTEIN KINASE C INHIBITORS

Protein kinase C (PKC) consists of a family of closely related enzymes that function as serine/threonine kinases. Protein kinase C plays an important role in cell-cell signaling, gene expression, and in the control of cell differentiation and growth. At present, there are currently at least ten known isozymes of PKC that differ in their tissue distribution, enzymatic specificity, and regulation.

10 Nishizuka Y. Annu. Rev. Biochem. 58: 31-44 (1989); Nishizuka Y. Science 258: 607-614 (1992).

Protein kinase C isozymes are single polypeptide chains ranging from 592 to 737 amino acids in length. The isozymes contain a regulatory domain and a catalytic domain connected by a linker peptide. The regulatory and catalytic domains can be further subdivided into constant and variable regions. The catalytic domain of protein kinase C is very similar to that seen in other protein kinases while the regulatory domain is unique to the PKC isozymes. The PKC isozymes demonstrate between 40-80% homology at the amino acid level among the group, however, the homology of a single isozyme between different species is generally greater than 97%.

Protein kinase C is a membrane-associated enzyme that is allosterically regulated by a number of factors, including membrane phospholipids, calcium, and certain membrane lipids such as diacylglycerols that are liberated in response to the activities of phospholipases. Bell, R.M. and Burns, D.J., J. Biol. Chem. 266: 4661-4664 (1991); Nishizuka, Y. Science 258: 607-614 (1992). The protein kinase C isozymes, alpha, beta-1, beta-2 and gamma, require membrane phospholipid, calcium and diacylglycerol/phorbol esters for full activation. The delta, epsilon, eta, and theta forms of PKC are calcium-independent in their mode of activation. The zeta and lambda forms of PKC are independent of both calcium and diacylglycerol and are believed to require only membrane phospholipid for their activation.

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Only one or two of the protein kinase C isozymes may be involved in a given disease state. For example, the elevated blood glucose levels found in diabetes lead to an isozyme-specific elevation of the beta-2 isozyme in vascular tissues. Inoguchi et al., Proc. Natl. Acad. Sci. USA 89: 11059-11065 (1992). A diabetes-linked elevation of the beta isozyme in human platelets has been correlated with their altered response to agonists. Bastyr III, E.J. and Lu, J. Diabetes 42: (Suppl 1) 97A (1993). The human vitamin D receptor has been shown to be selectively phosphorylated by protein kinase C beta. This phosphorylation has been linked to alterations in the functioning of the receptor. Hsieh et al., Proc. Natl. Acad. Sci. USA 88: 9315-9319 (1991); Hsieh et al., J. Biol. Chem. 268: 15118-15126 (1993). In addition, recent work has shown that the beta-2 isozyme is responsible for erythroleukemia cell proliferation while the alpha isozyme is involved in megakaryocyte differentiation in these same cells. Murray et al., J. Biol. Chem. 268: 15847-15853 (1993).

The ubiquitous nature of the protein kinase C isozymes and their important roles in physiology provide incentives to produce highly isozyme selective PKC inhibitors. Given the evidence demonstrating linkage of certain isozymes to disease states, it is reasonable to assume that inhibitory compounds that are selective to one or two protein kinase C isozymes relative to the other PKC isozymes are superior therapeutic agents. Such compounds should demonstrate greater efficacy and lower toxicity by virtue of their specificity.

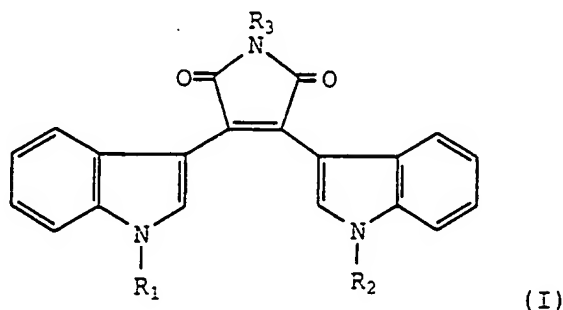
The art recognizes various classes of compounds as protein kinase C inhibitors. Some of these compounds are also known to demonstrate specificity to protein kinase C. However, very little is known regarding isozyme selectivity. Studies of the PKC-selective compound, 3-[1-(3-dimethylaminopropyl)-indol-3-yl]-4-(1H-indol-3-yl)-1H-pyrrole-2,5-dione, suggest a slight selectivity for the calcium dependent isozymes, but find no isozyme selectivity

- 3 -

between alpha, beta-1, beta-2, and gamma. Toullec et al., J. Biol. Chem. 266: 15771-15781 (1991). Martiny-Baron, et al., J. Biol. Chem. 268: 9194-9197 (1993), tested the same compound and found slight selectivity for isozymes, alpha and beta versus delta, epsilon, and zeta. Martiny-Baron observed no differences in the selectivity between alpha and beta-1 isozymes. Wilkinson, et al., Biochem. J. 294: 335-337 (1993), failed to observe any high degree of isozyme selectivity and suggest only slight selectivity for the alpha isozyme and equal inhibition of beta, gamma, and epsilon for several species of bis-indolemaleimides. Therefore, despite years of research, there remains a need for therapeutically effective isozyme-selective inhibitors.

This invention provides compounds that are highly isozyme selective. The compounds selectively inhibit protein kinase C beta-1 and beta-2 isozymes. Accordingly, the present invention also provides a method of selectively inhibiting protein kinase C isozymes beta-1 and beta-2. As isozyme selective inhibitors of beta-1 and beta-2, the compounds are therapeutically useful in treating conditions associated with diabetes mellitus and its complications, as well as other disease states associated with an elevation of the beta-1 and beta-2 isozymes.

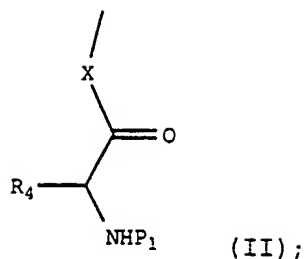
This invention provides compounds, which selectively inhibit protein kinase C beta-1 and beta-2 isozyme, of the Formula I:



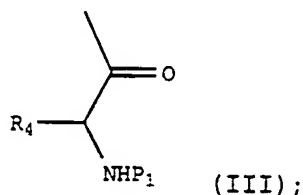
wherein:

R₁ is of the Formula II:

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R_2 is hydrogen, alkyl, acyl, alkoxyalkyl, hydroxyalkyl, aminoalkyl, monoalkylaminoalkyl, dialkylaminoalkyl, acylaminoalkyl, N_3 -alkyl, or an amino acid of the Formula (III):



R_3 is H or CH_3 ;

10

R_4 is an amino acid side chain;

X is $-(CH_2)_n-NH-$, $-(CH_2)_n-O-$, phenylene- $NH-$, phenylene- $O-$, or a bond;

15

P_1 is H, alkyl, or an amino protecting group; and

n is 1, 2 or 3.

20

The invention further provides a method of selectively inhibiting protein kinase C beta-1 and beta-2 isozyme, which comprises administering to a mammal in need of such treatment a pharmaceutically effective amount of a compound of the Formula I. As selective inhibitors, the invention further provides a method for treating diabetes mellitus, which comprises administering to a mammal in need of such treatment a pharmaceutically effective amount of a compound of the Formula I. The invention also provides

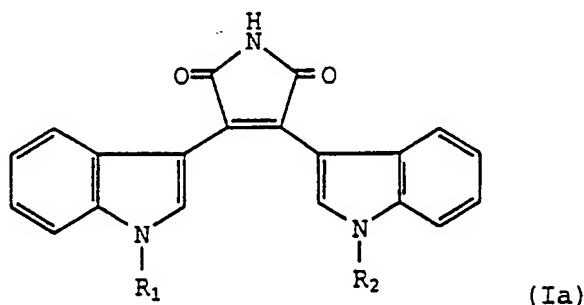
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pharmaceutical formulations comprising a compound of the Formula I associated with one or more pharmaceutically acceptable excipients, carriers, or diluents.

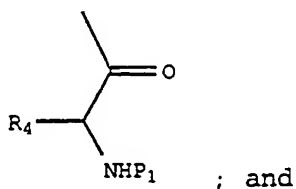
- 5 As noted above, the invention provides compounds of the Formula I which selectively inhibit isozymes of protein kinase C.

The preferred compounds are compounds of the Formula Ia:

10

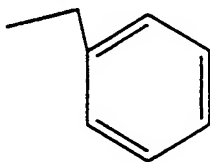


wherein: R₁ is



- 15 R₂ is amino alkyl, monoalkylamino alkyl, or dialkylamino alkyl.

Particularly preferred compounds are those wherein R₄ is H, CH₃, CH₂CH(CH₃)₂, or



- 20 and P₁ is t-butoxycarbonyl (BOC) or benzyloxycarbonyl (CBZ).

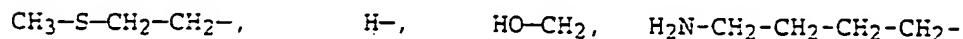
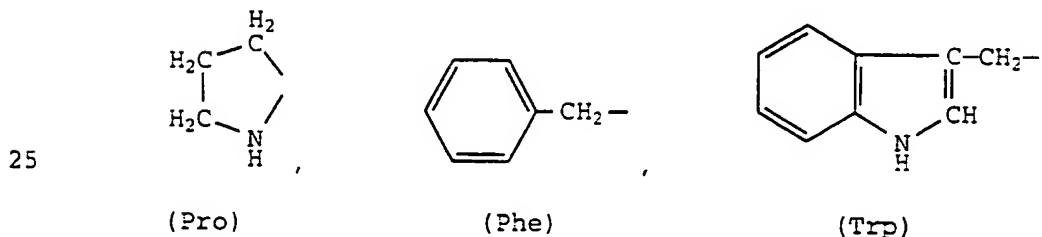
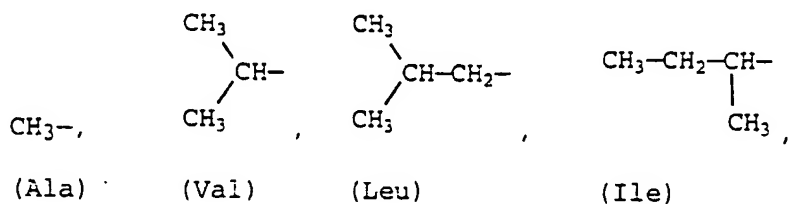
As used herein, the term "alkyl", alone or in combinations, means a straight or branched-chain alkyl group containing from one to seven, preferably one to four, carbon

atoms such as methyl, ethyl, propyl, isopropyl, butyl, sec-butyl, t-butyl and pentyl. The term "C₁₋₄ alkyl" is an alkyl limited to one to four carbon atoms.

The term "alkoxy", alone or in combinations, is an alkyl covalently bonded to the parent moiety by a -O-linkage. Examples of alkoxy groups are methoxy, ethoxy, propoxy, isopropoxy, butoxy and t-butoxy. An alkoxyalkyl is, for example, CH₃(CH₂)_z-O-(CH₂)_z- wherein z is from one to seven or preferably one to four.

The acyl moiety, alone or in combination, is derived from an alkanoic acid containing a maximum of seven, preferably a maximum of four, carbon atoms (e.g. acetyl, propionyl or butyryl) or from an aromatic carboxylic acid (e.g. benzoyl). An acylamino is, for example, CH₃(C=O)NH-(acetylamino). Likewise, an acylaminoalkyl is CH₃(C=O)NH(CH₂)_z- wherein z is from one to seven or preferably one to four.

The term "amino acid side chain" represents the variable region of the naturally occurring amino acids, which are of the formulas:



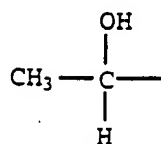
- 7 -

(Met)

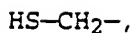
(Gly)

(Ser)

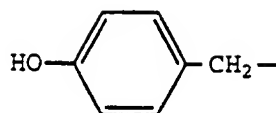
(Lys)



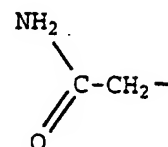
(Thr)



(Cys)

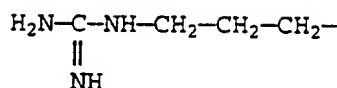


(Tyr)

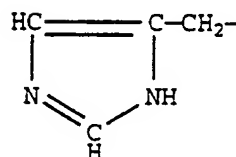


(Asn)

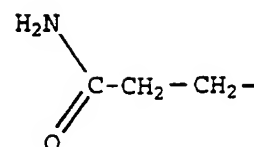
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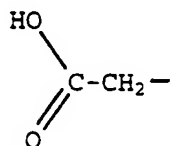
(Arg)



(His)

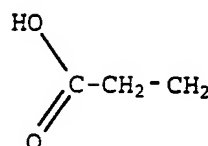


(Gln)



(Asp)

, or



(Glu)

10

The term "amino protecting group" as used herein refers to substituents commonly employed to block or protect the amino functionality. Preferred amino-protecting groups are t-butoxycarbonyl and benzyloxycarbonyl. Other amino protecting groups are found in J. W. Barton, Protective Groups in Organic Chemistry, J.G.W. McOmie, Ed., Plenum Press, New York, N.Y., 1973, Chapter 2, and T. W. Greene, Protective Groups in Organic Synthesis, John Wiley and Sons, New York, N.Y., 1981, Chapter 7, herein incorporated by reference. The related term "protected amino" defines an amino group substituted with an amino protecting group as previously discussed.

The term "pharmaceutically effective amount", as used herein, represents an amount of a compound of the

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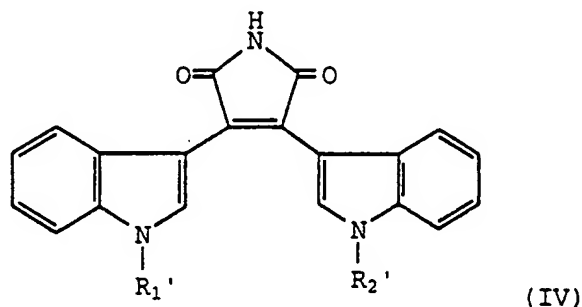
invention that is capable of selectively inhibiting PKC isozyme activity in mammals. The particular dose of the compound administered according to this invention will, of course, be determined by a physician under the particular
5 circumstances surrounding the case, including the compound administered, the route of administration, the particular condition being treated, and similar considerations. The compounds can be administered by a variety of routes including the oral, rectal, transdermal, subcutaneous,
10 topical, intravenous, intramuscular or intranasal routes.

The term "treating," as used herein, describes the management and care of a patient for the purpose of combating the disease, condition, or disorder and includes the administration of a compound of present invention to prevent
15 the onset of the symptoms or complications, alleviating the symptoms or complications, or eliminating the disease, condition, or disorder.

The term "isozyme selective" means the preferential inhibition of protein kinase C beta-1 or beta-2 isozymes over
20 protein kinase C isozymes, alpha, gamma, delta, epsilon, zeta, and eta. In general, the compounds demonstrate a minimum of a eight fold differential, preferably a ten fold differential, in the dosage required to inhibit PKC beta-1 or beta-2 isozymes and the dosage required for equal inhibition
25 of the alpha protein kinase C isozyme as measured in the PKC assay. The compounds demonstrate this differential across the range of inhibition and are exemplified at the IC₅₀, i.e., a 50% inhibition. Accordingly, the invention provides a method for selectively inhibiting the beta-1 or beta-2
30 protein kinase C isozyme. A related phrase is "selectively inhibiting protein kinase C beta-1 and beta-2 isozymes," which refers to isozyme selective inhibition. Thus, because one needs a substantially higher concentration of compound to inhibit the other protein kinase C isozymes (e.g., Example 1
35 discloses 50% inhibition at a concentration of 0.031 $\mu\text{mol/L}$ for the beta-2 isozyme while the IC₅₀ with respect to the alpha protein kinase C isozyme is 1.4 $\mu\text{mol/L}$) a

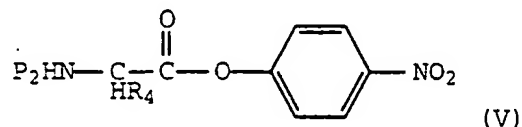
pharmaceutically effective dosage of the compound inhibits beta-1 and beta-2 protein kinase C isozymes with lower toxicity by virtue of their minimal inhibition of the other isozymes. Surprisingly, the bis-indolyl maleimide containing two amino acids (i.e., R₁ is of the Formula II and R₂ is of the Formula III) are yellow in appearance. This is a marked advantage because a yellow compound does not interfere with testing or monitoring of the urine of patients taking the drug.

The preparation of bis-indolylmaleimides of the Formula:



- wherein R₁' is hydrogen, and R₂' is hydrogen, alkyl, alkoxyalkyl, hydroxyalkyl, aminoalkyl, monoalkylaminoalkyl, dialkylaminoalkyl, or acylaminoalkyl, is disclosed in U.S. Patent 5,057,614 and known in the art as evidenced by EPO 397 060 (1990) and Bit et al., *J. Med. Chem.* **36**: 21-29 (1993). U.S. Patent 5,057,614 is herein incorporated by reference.

The compounds of Formula I, are prepared by reacting a compound of Formula IV with an activated amino acid of the Formula V:



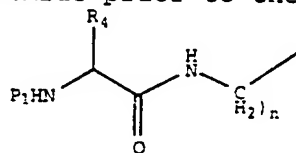
wherein P₂ is an amino protecting group; and R₄ is an amino acid side chain.

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The acylation of Compound IV with the activated amino acid (Compound V) is carried out in acetonitrile in the presence of 18-crown-6 and fluorine anion under basic conditions. The compounds containing an amine (e.g. Example 2) are prepared preferably with pyridine as the base for solubility purposes. The preferred base when the solubility of the substrate is not important is diisopropylethylamine. The reaction conditions and other acceptable solvents are known in the art and described in Klausner, et al., J. Chem. Soc. Perkin I: 607-631 (1977); and Nakagawa, et al., J. Am. Chem. Soc., 105: 3709-3710 (1983).

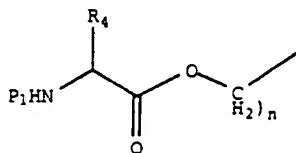
The compounds of Formula V are commercially available or can be prepared by known methodology, e.g., Wolman Y et al, J. Chem. Soc. C: 689 (1967); Bodanszky M et al, J. Amer. Chem. Soc., 81: 2504 (1959); Sakakibara S et al, Bull. Chem. Soc. Jpn., 37: 1231 (1964)

When preparing the compounds of Formula I, wherein X is $-(CH_2)_n-NH-$, $-(CH_2)_n-O-$, phenylene- $NH-$, or phenylene- $O-$, the linking moiety, X, is appended to the bis-indolylmaleimide prior to the coupling. Thus, for example,



when R_1 is

, R_1' is aminoalkyl. Likewise,



when R_1 is

, R_1' is hydroxyalkyl. The

coupling reaction when X is $-(CH_2)_n-O-$ or phenylene- $O-$ may be alternatively carried out with dicyclohexylcarbodiimide and 4-dimethylaminopyridine under standard coupling conditions known in the art.

By virtue of their acidic moieties, the compounds of Formula I include the pharmaceutically acceptable base addition salts thereof. Such salts include those derived from inorganic bases such as ammonium and alkali and alkaline earth metal hydroxides, carbonates, bicarbonates, and the

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like, as well as salts derived from basic organic amines such as aliphatic and aromatic amines, aliphatic diamines, hydroxyalkamines, and the like. Such bases useful in preparing the salts of this invention thus include ammonium hydroxide,
5 potassium carbonate, sodium bicarbonate, calcium hydroxide, methylamine, diethylamine, ethylenediamine, cyclohexylamine, ethanolamine and the like.

Because of the basic moiety, the compounds of Formula I can also exist as pharmaceutically acceptable acid
10 addition salts. Acids commonly employed to form such salts include inorganic acids such as hydrochloric, hydrobromic, hydroiodic, sulfuric and phosphoric acid, as well as organic acids such as para-toluenesulfonic, methanesulfonic, oxalic, para-bromophenylsulfonic, carbonic, succinic, citric,
15 benzoic, acetic acid, and related inorganic and organic acids. Such pharmaceutically acceptable salts thus include sulfate, pyrosulfate, bisulfate, sulfite, bisulfite, phosphate, monohydrogenphosphate, dihydrogenphosphate, metaphosphate, pyrophosphate, chloride, bromide, iodide,
20 acetate, propionate, decanoate, caprylate, acrylate, formate, isobutyrate, caproate, heptanoate, propiolate, oxalate, malonate, succinate, suberate, sebacate, fumarate, maleate, 2-butyne-1,4-dioate, 3-hexyne-2,5-dioate, benzoate, chlorobenzoate, hydroxybenzoate, methoxybenzoate, phthalate,
25 xylenesulfonate, phenylacetate, phenylpropionate, phenylbutyrate, citrate, lactate, hippurate, β -hydroxybutyrate, glycollate, maleate, tartrate, methanesulfonate, propanesulfonate, naphthalene-1-sulfonate, naphthalene-2-sulfonate, mandelate and the like salts.

30 In addition to pharmaceutically-acceptable salts, other salts are included in the invention. They may serve as intermediates in the purification of compounds or in the preparation of other salts, or are useful for the identification, characterization or purification.

35 The pharmaceutically acceptable salts of compounds of Formula I can also exist as various solvates, such as with water, methanol, ethanol, dimethylformamide, ethyl acetate

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and the like. Mixtures of such solvates can also be prepared. The source of such solvate can be from the solvent of crystallization, inherent in the solvent of preparation or crystallization, or adventitious to such solvent. Such
5 solvates are within the scope of the present invention.

It is recognized that various stereoisomeric forms of the compounds of Formula I may exist; for example, R₁ introduces a chiral carbon atom. The compounds are normally prepared as racemates and can conveniently be used as such,
10 but individual enantiomers can be isolated or synthesized by conventional techniques if so desired. Such racemates and individual enantiomers and mixture thereof form part of the present invention.

The following examples are provided merely to
15 further illustrate the invention. The scope of the invention is not construed as merely consisting of the following examples. In the following examples and preparations, melting point, nuclear magnetic resonance spectra, mass spectra, high pressure liquid chromatography, N,N-dimethylformamide, palladium on charcoal, diisobutylaluminum
20 hydride, acetonitrile, and tetrahydrofuran are abbreviated M.Pt., NMR, MS, HPLC, DMF, Pd/C, DIBAL, ACN and THF, respectively. The terms "NMR" and "MS" indicate that the spectrum was consistent with the desired structure.

25

Example 1

3-[1-(3-azidopropyl)-3-indolyl]-4-[(1-N-^tBOC)-glycine-3-indolyl]-1H-pyrrol-2,5-dione

30

3-[1-(3-azidopropyl)-3-indolyl]-4-[3-indolyl]-1H-pyrrol-2,5-dione (0.20 g, 0.49 mol., 1 eq.) was transferred to an oven dried 50 mL round bottom flask equipped with a stir bar, septum, and N₂ balloon. The red solid was
35 dissolved in about 25 mL of anhydrous acetonitrile (delivered via canula) and stirring started. To the red solution was added (N-^tBOC)glycine, p-nitrophenyl ester (0.22 g, 0.74

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mmol, 1.5 eq), 18-crown-6 (0.13 g, 0.49 mmol, 1 eq), and N,N-diisopropylethylamine (0.08 g, 0.61 mmol, 1.25 eq, 0.11 mL), and potassium fluoride (0.06 g, 0.98 mmol, 2 eq) with vigorous stirring. This red solution was allowed to stir under a N₂ atmosphere for 5 days.

TLC (50% EtOAc in hexanes) of the reaction indicated total loss of starting materials. The reaction was diluted with 100 mL of EtOAc, transferred to a separatory funnel, washed with water, and brine. The organic layer was dried over MgSO₄. The solvent was removed in vacuo to obtain a red oil. This oil was purified by silica flash chromatography using 37.5% EtOAc in hexanes as the mobile phase to give 0.2055 g of an orange solid.

NMR, MS

15 Elemental Analysis:

Theory:	C 63.48	N 17.27	H 5.15
Found:	C 63.53	N 16.28	H 5.64

Example 2

3-[1-(3-N,N'-dimethylaminopropyl)-3-indolyl]-4-[(1-N-^tBOC)-glycine-3-indolyl]-1H-pyrrol-2,5-dione

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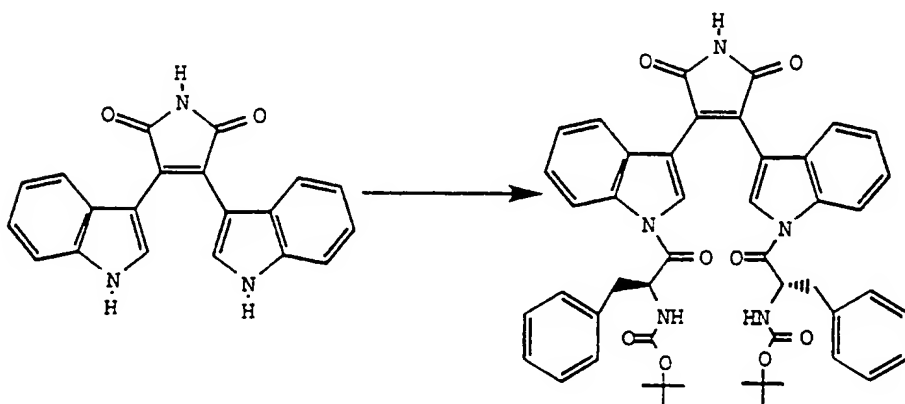
3-[1-(3-N,N'-dimethylaminopropyl)-3-indolyl]-4-[3-indolyl]-1H-pyrrol-2,5-dione (0.05 g, 0.12 mmol, 1 eq) was transferred to a 100 mL round bottom flask equipped with a stir bar, septum, and N₂ balloon. Anhydrous acetonitrile (70 mL) was delivered via canula followed by pyridine (0.05 g, 0.62 mmol, 5 eq, 0.05 mL) via syringe. The resulting orange suspension was heated to 50°C for 60 minutes to help solubilize the compound. After 60 minutes, (N-^tBOC)glycine, *p*-nitrophenyl ester (0.07 g, 0.24 mmol, 2 eq), 18-crown-6 (0.06 g, 0.24 mmol, 2 eq), and dry potassium fluoride (0.03 g, 0.48 mmol, 4 eq) were added as solids with vigorous stirring. The resulting yellow/orange solution was allowed to stir at room temperature under an atmosphere of N₂ for 48 hours. MS.

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Proton NMR (300 MHz in CDCl₃): 1.48 (s, 9H, t-butyl grp of BOC), 2.4 (2H, NCH₂CH₂CH₂N(CH₃)₂), 2.95 (6H, -N(CH₃)₂), 3.25 (2H, CH₂N(CH₃)₂), 3.7 (2H, C(=O)CH₂NHBOC), 4.55 (2H, NCH₂CH₂CH₂N(CH₃)₂), 5.18 (1H, NHBOC), 6.57-7.4 (10H, aromatics), 8.2 (d, J=9 Hz, 1H, C2' proton of acylated indole), 8.6 (1H, maleimide NH).

Example 3

3,4-Bis[(1-N-^tBOC)-phenylalanine-3-indolyl]-1H-pyrrol-2,5-dione



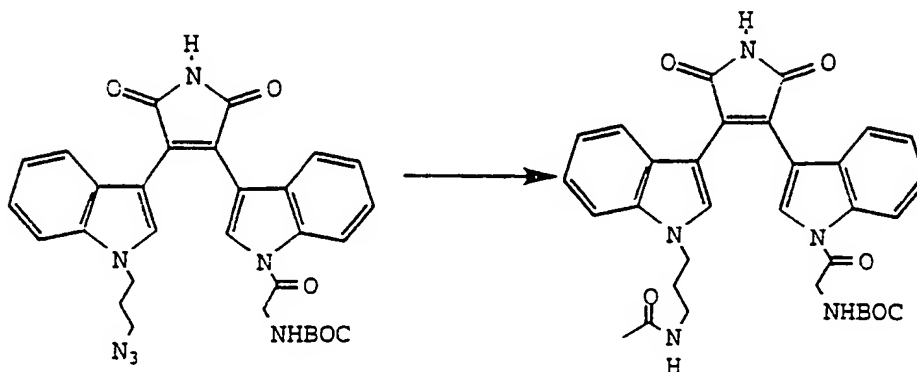
3,4-Bis[3-indolyl]-1H-pyrrol-2,5-dione (0.165 g, 0.5 mmol, 2 eq) was transferred to an oven dried 50 mL round bottom flask equipped with stir bar, septum, and N₂ balloon. Anhydrous acetonitrile (25 mL) was added via canula and stirring started to produce a red solution. (N-^tBOC)phenylalanine, p-nitrophenyl ester (0.67 g, 1.75 mmol, 3.5 eq), 18-crown-6 (0.13 g, 0.5 mmol, 2 eq), and diisopropylethylamine (0.16 g, 1.25 mmol, 2.5 eq, 0.22 mL) were then added to the solution with vigorous stirring. When everything had dissolved, anhydrous potassium fluoride (0.12 g, 2 mmol, 4 eq) was added as a solid. The solution was allowed to stir under nitrogen at room temperature overnight. After 16 hours of stirring, TLC (50% EtOAc in hexanes) showed loss of starting material. The yellow solution was transferred to a separatory funnel with ~150 mL of EtOAc. This solution was washed with water and brine.

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The organic layer was collected and then dried over MgSO_4 . The solvent was removed to give a bright yellow/orange residue. This residue was purified by silica flash chromatography using 37.5% EtOAc in hexanes as the mobile phase to give a bright yellow film after the solvent was removed. HPLC of this solid still showed some impurities so the product was purified using the gel permeation hydrophobic columns with CHCl_3 as the mobile phase to give 229 mg of a bright yellow solid. NMR, MS (FD (in MeOH): MW-821.94; m/z 822 (MH+).

Example 4

3-[1-(3-acetamidopropyl)-3-indolyl]-4-[(1-N- t BOC)-glycine-3-indolyl]-1H-pyrrol-2,5-dione



3-[1-(3-azidopropyl)-3-indolyl]-4-[(1-N- t BOC)-glycine-3-indolyl]-1H-pyrrol-2,5-dione (80 mg, 0.14 mmol, 1 eq) was dissolved in ethyl acetate in a 100 mL round bottom flask equipped with a stir bar and 14/22 adapter. Lindlar's catalyst (catalytic, 8 mg) and acetic anhydride (40 mg, 0.39 mmol, 3 eq) were added with vigorous stirring to the red solution. A hydrogen balloon was placed on top of the flask. The flask was then evacuated and filled with hydrogen 3 times to remove dissolved oxygen from the solution. The reaction was allowed to stir at room temperature under the hydrogen atmosphere overnight.

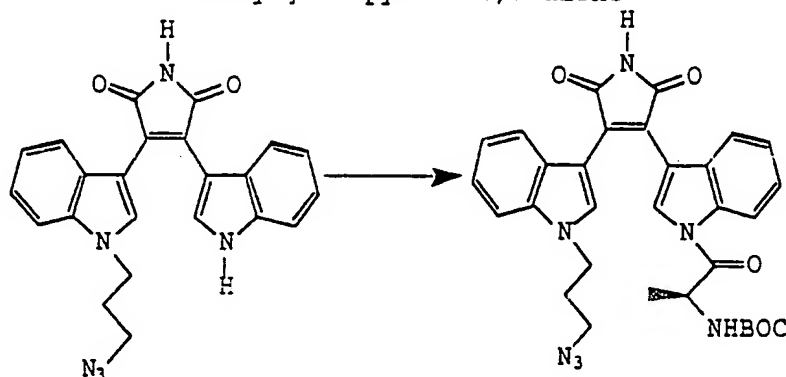
After stirring overnight, TLC (50% EtOAc in hexanes) of the reaction indicated loss of the starting

- 16 -

material. The reaction was filtered through a pad of celite to remove the hydrogenation catalyst. The red solution was transferred to a separatory funnel and diluted with 100 mL of EtOAc. The organic layer was washed with water and brine and then collected and dried over MgSO₄. The solvent was removed to give an orange residue. This residue was purified using size exclusion gel permeation columns using chloroform as the mobile phase to give 52.4 mg of an orange residue. MS (FD (in MeOH): MW=583.65; m/z 584 (MH⁺), 426 (M⁺-amino acid).

Example 5

3-[1-(3-azidopropyl)-3-indolyl]-4-[(1-N^tBOC)-alanine-3-indolyl]-1H-pyrrol-2,5-dione



15

The azide (0.36 g, 0.88 mmol, 1 eq) was transferred to a dried 100 mL round bottom flask equipped with stir bar, septum, and N₂ balloon. Anhydrous acetonitrile was transferred to the flask via canula and stirring started. 18-Crown-6 (0.23 g, 0.88 mmol, 1 eq) and (N-BOC)alanine, p-nitrophenyl ester (0.48 g, 1.54 mmol, 1.75 eq) were added as solids and diisopropylethylamine (0.14 g, 1.10 mmol, 1.25 eq) was added via syringe to the vigorously stirred red solution. The red solution was allowed to stir under N₂ overnight.

After stirring for 16 hours, TLC (37.5% EtOAc in hexanes) showed the loss of the starting material. The reaction was diluted with EtOAc and transferred to a

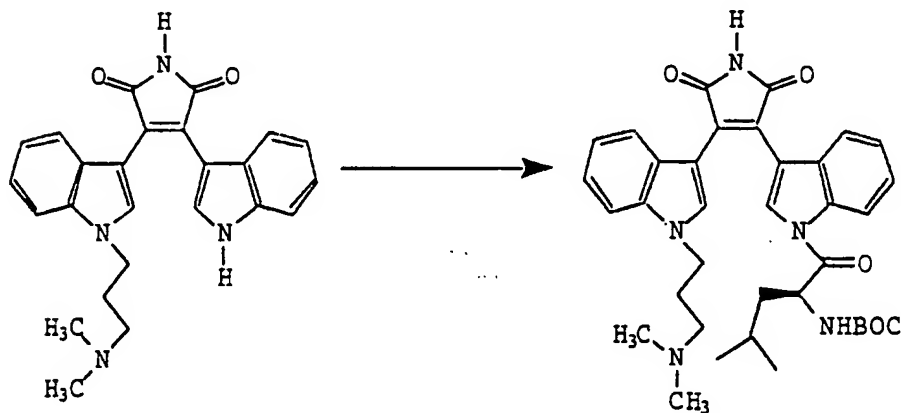
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separatory funnel and washed with water and brine. The organic layer was collected, dried over MgSO_4 , and the solvent removed. The resulting orange oil was purified using a silica gel flash column using 37.5% EtOAc in hexanes as the mobile phase to obtain 0.42 g (82% yield) of a red/orange solid.

(300 MHz in CDCl_3): 1.42 (d, $J=6$ Hz, 3H, methyl grp of alanine side chain), 1.5 (s, 9H, t-butyl group of BOC protecting group), 2.0 (m, 2H, $\text{NCH}_2\text{CH}_2\text{CH}_2\text{N}_3$), 3.2 (m, 2H, $\text{NCH}_2\text{CH}_2\text{CH}_2\text{N}_3$), 4.2 (m, 2H, $\text{NCH}_2\text{CH}_2\text{CH}_2\text{N}_3$), 5.13 (m, 1H, α proton of alanine), 6.8-8.4 (m, 10H, aromatics).

Example 6

3-[1-(3-N,N'-dimethylaminopropyl)-3-indolyl]-4-[3-indolyl]-1H-pyrrol-2,5-dione



3-[1-(3-N,N'-dimethylaminopropyl)-3-indolyl]-4-[3-indolyl]-1H-pyrrol-2,5-dione (0.11 g, 0.27 mmol, 1 eq) was transferred to a dry 100 mL round bottom flask equipped with stir bar, septum, and N_2 balloon. Anhydrous acetonitrile (25 mL) was delivered via canula and stirring started. To the red/orange suspension was added pyridine (0.11 g, 1.35 mmol, 5 eq) via syringe. 18-Crown-6 (0.14 g, 0.54 mmol, 2 eq) and (N-BOC)leucine p-nitrophenylester (0.19 g, 0.54 mmol, 2 eq) were then added as solids to the suspension. Dry KF (0.06 g, 1.08 mmol, 4 eq) was then quickly added as a

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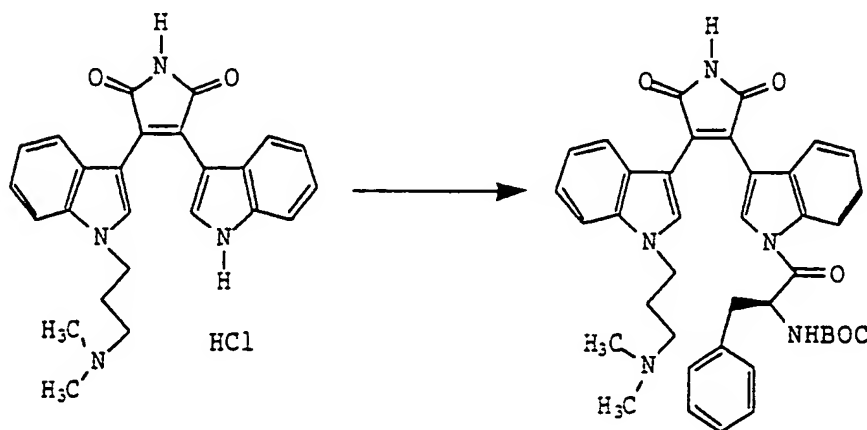
solid with vigorous stirring. Vigorous stirring was continued under N₂ at 50°C using a water bath. (After about 3 hours, the suspension became a red/orange solution.)

After 3 days, TLC (10% MeOH in acetone) showed loss of starting material. The reaction was diluted with EtOAc and washed with water and then brine. The organic layer was collected, dried over MgSO₄, and the solvent removed to give a red/orange oil. This oil was purified by flash chromatography (silica gel) using 10% MeOH in acetone as the mobile phase to give 0.11 g of a reddish solid. MS.

Proton NMR (300 MHz in d₆-DMSO): 0.70 and 0.76 (d, 3H each, J=6.6 Hz, the -CH₃ groups of the leucine side chain), 0.83 (t, J=7.2 Hz, 1H, CH of leucine side chain), 1.17 (s, 6H, N(CH₃)₂), 1.39 (s, 9H, BOC group), 1.59 (m, 2H, beta H on leucine side chain), 1.84 (m, 2H, NCH₂CH₂CH₂N), 2.17 (m, 2H, NCH₂CH₂CH₂N(CH₃)₂), 4.28 (m, 2H, NCH₂CH₂CH₂N(CH₃)₂), 4.56 (br, 1H, NHBOC), 4.77 (m, 1H, alpha proton of leucine side chain), 6.6-8.2 (m, 10H, aromatic protons), 11.2 (s, 1H, maleimide NH).

Example 7

3-[1-(3-N,N'-dimethylaminopropyl)-3-indolyl]-4-[3-indolyl]-1H-pyrrol-2,5-dione hydrochloride



3-[1-(3-N,N'-dimethylaminopropyl)-3-indolyl]-4-[3-indolyl]-1H-pyrrol-2,5-dione hydrochloride (0.25 g, 0.56

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mmol, 1 eq) was suspended in 25 mL of anhydrous acetonitrile in a dry 100 mL round bottom flask equipped with stir bar, septum, and N₂ balloon. The 18-crown-6 (0.30 g, 1.12 mmol, 2 eq) and the (N-BOC)phenylalanine, p-nitrophenyl ester
5 (0.43 g, 1.12 mmol, 2 eq) were added as solids followed by pyridine (0.27 g, 3.36 mmol, 6 eq) via syringe. Anhydrous KF was added as a solid to the vigorously stirred suspension. The temperature of the flask was raised to 55°C with a water bath and the suspension was allowed to stir
10 overnight under a nitrogen atmosphere at 55°C (after about 3 hours the suspension turned into a solution).

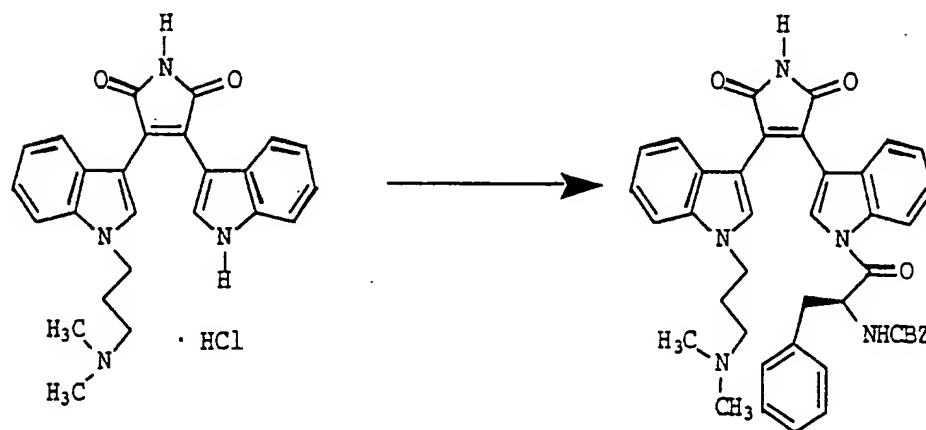
After 36 hours of stirring at 55°C, the reaction was diluted with 300 mL of ethyl acetate and transferred to a separatory funnel and washed with water and then brine. The
15 organic layer was collected, dried over MgSO₄, and the solvent removed on the rotary evaporator. The orange oil was purified by flash chromatography on silica gel using 10% MeOH in acetone as the mobile phase. Removal of the solvent gave 0.13 g (39%) of an orange solid. MS.

20 Carbon NMR (75 MHz in CDCl₃): 26.4, 28.3, 28.5, 29.3, 31.7, 38.8, 44.2, 44.3, 53.8, 54.2, 55.5, 80.3, 105.4, 110.0, 113.7, 116.5, 121.0, 121.8, 122.0, 122.8, 123.8, 124.0, 125.6, 125.7, 126.2, 126.5, 127.1, 128.0, 128.3, 128.4, 129.5, 133.0, 133.3, 135.2, 135.6, 136.3, 170.2,
25 171.6, 172.0

- 20 -

Example 8

3-[1-(3-N,N'-dimethylaminopropyl)-3-indolyl]-4-[3-indolyl]-
1H-pyrrol-2,5-dione hydrochloride



5

3-[1-(3-N,N'-dimethylaminopropyl)-3-indolyl]-4-[3-indolyl]-1H-pyrrol-2,5-dione hydrochloride (0.25 g, 0.56 mmol, 1 eq) was transferred to a dry 100 mL round bottom flask equipped with stir bar, septum, and N₂ balloon. Anhydrous acetonitrile (25 mL) was delivered to the round bottom via canula followed by pyridine (0.27 g, 3.36 mmol, 6 eq) via syringe. 18-Crown-6 (0.30 g, 1.12 mmol, 2 eq), (N-CBZ)phenylalanine p-nitrophenylester (0.47 g, 1.12 mmol, 2 eq), and KF (0.13 g, 2.24 mmol, 4 eq) were added as solids to the vigorously stirred suspension. The suspension was heated to 50°C with a water bath. (The suspension turned into a red/orange solution after about 3 hours of stirring.) The solution was allowed to stir at 50°C for 4 days.

After 4 days, TLC (10% MeOH in acetone) showed no starting material. The reaction was diluted with EtOAc and transferred to a separatory funnel. It was washed with water and brine. The organic layer was collected, dried over MgSO₄, and the solvent removed to give an orange oil. This oil was purified by silica gel flash chromatography using 10% MeOH in acetone as the mobile phase. The resulting orange solid still contained some impurities and

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was purified using gel filtration chromatography using CHCl_3 as the mobile phase. MS.

Carbon NMR (75 MHz in CDCl_3): 24.7, 43.4, 43.7, 45.8, 55.2, 66.8, 110.1, 113.3, 133.4, 116.4, 121.2, 121.7, 122.0, 5 122.8, 123.9, 125.7, 126.6, 127.0, 127.6, 127.7, 127.8, 127.9, 128.0, 128.1, 128.3, 128.4, 128.6, 129.6, 129.8, 134.4, 134.6, 135.0, 136.0, 154.9, 172.0

As previously noted, the compounds of the present 10 invention are potent, beta-1 and beta-2 isozyme selective PKC inhibitors. As such, they are useful in the treatment of conditions associated with diabetes mellitus and its complications, as well as other disease states associated with an elevation of the beta-1 and beta-2 isozymes.

15 Protein kinase C beta-1 and beta-2 has been linked to diabetes. Inoguchi et al., Proc. Natl. Acad. Sci. USA 89: 11059-11065 (1992). Excessive activity of protein kinase C has been linked to insulin signaling defects and therefore to the insulin resistance seen in Type II 20 diabetes. Karasik, A. et al., J. Biol. Chem. 265: 10226-10231 (1990); Chen, K.S. et al., Trans. Assoc. Am. Physicians 104: 206-212 (1991); Chin, J.E. et al., J. Biol. Chem. 268: 6338-6347 (1993). Further, studies have demonstrated a marked increase in protein kinase C activity 25 in tissues known to be susceptible to diabetic complications when exposed to hyperglycemic conditions. Lee, T.-S. et al., J. Clin. Invest. 83: 90-94 (1989); Lee, T.-S. et al., Proc. Natl. Acad. Sci. USA 86: 5141-5145 (1989); Craven, P.A. and DeRubertis, F.R. J. Clin. Invest. 83: 1667-1675 30 (1989); Wolf, B.A. et al., J. Clin. Invest. 87: 31-38 (1991); Tesfamariam, B. et al., J. Clin. Invest. 87: 1643-1648 (1991); Bastyr III, E.J. and Lu, J., Diabetes 42: (Suppl 1) 97A (1993).

The ability of the compounds of the present 35 invention to selectively inhibit protein kinase C beta-1 and beta-2 isozyme was determined in the PKC Enzyme assay.

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PKC Enzyme Assay

PKC enzymes = alpha, beta I, beta II, gamma, delta, epsilon, eta and zeta.

Assay components in a total volume of 250 μ L are the following:

Vesicles consisting of 120 μ g/mL phosphatidylserine (Avanti Polar Lipids) and sufficient diacylglycerol (Avanti Polar Lipids) to activate the enzyme to maximum activity in 20 mM HEPES buffer (Sigma, St. Louis, Missouri), pH 7.5, 940 μ M calcium chloride (Sigma, St. Louis, Missouri) for assaying the alpha, beta I, beta II and gamma enzyme only, 1 mM EGTA for all the enzymes, 10 mM magnesium chloride (Sigma, St. Louis, Missouri) and 30 μ M (gamma-32P) ATP (DuPont). For all the enzymes either histone type HL (Worthington) or myelin basic protein is used as substrate. The assay is started by addition of protein kinase C enzyme incubated at 30° C for 10 minutes and stopped by adding 0.5 mL of cold trichloroacetic acid (Amresco) followed by 100 μ L of 1 mg/mL bovine serum albumin (Sigma, St. Louis, Missouri). The precipitate is collected by vacuum filtration on glass fiber filters employing a TOMTEC™ filtration system and quantified by counting in a beta scintillation counter.

Using the methodology described, representative compounds were evaluated and were found to have an IC₅₀ value with respect to the beta-1 and beta-2 isozyme of below 10 μ M. The compounds are isozyme selective, i.e., the compounds preferentially inhibit protein kinase C beta-1 and beta-2 isozyme over the protein kinase C isozymes, alpha, gamma, delta, epsilon, zeta, and eta. In general, the compounds demonstrate a minimum of an eight fold differential in the dosage required to inhibit PKC beta-1 or beta-2 isozyme and the dosage required for equal inhibition of the alpha protein kinase C isozyme as measured in this assay. Therefore, as selective inhibitors of PKC isozyme beta-1 and beta-2, the compounds are useful in the treatment of conditions in which PKC beta has demonstrated a role in

the pathology, in particular, diabetes mellitus and its complications.

Table 1 demonstrates the activity of several representative compounds.

5

Table 1

IC₅₀ (μm)

Ex.	α	β1	β2	γ	δ	ε	ζ	η
1	1.4	0.26	0.031	19	4.6	NA	100	2.6
2	2.3	0.15	0.04	2.6	2.9	6.1	45	0.37
3	83	8	2.6	>100	>100	NA	>100	NA

NA - data not available

10

The compounds of Formula I are formulated prior to administration. A pharmaceutical formulation comprises a compound of the Formula I with one or more pharmaceutically acceptable excipients, carriers, or diluents.

15 Pharmaceutical formulations are prepared by known procedures using well known and readily available ingredients. In making the compositions, the active ingredient will usually be mixed with a carrier, or diluted by a carrier, or enclosed within a carrier, which may be in the form of a
20 capsule, sachet, paper or other container. When the carrier serves as a diluent, it may be a solid, semisolid or liquid material which acts as a vehicle, excipient or medium for the active ingredient. Thus, the compositions can be in the form of tablets, pills, powders, lozenges, sachets, cachets,
25 elixirs, suspensions, emulsions, solutions, syrups, aerosol (as a solid or in a liquid medium), soft and hard gelatin capsules, suppositories, sterile injectable solutions and sterile packaged powders.

Some examples of suitable carriers, excipients,
30 and diluents include lactose, dextrose, sucrose, sorbitol, mannitol, starches, gum acacia, calcium phosphate, alginates, tragacanth, gelatin, calcium silicate, microcrystalline cellulose, polyvinylpyrrolidone, cellulose,

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water syrup, methyl cellulose, methyl and propylhydroxybenzoates, talc, magnesium stearate and mineral oil. The formulations can additionally include lubricating agents, wetting agents, emulsifying and suspending agents, preserving agents, sweetening agents or flavoring agents. The compositions of the invention may be formulated so as to provide quick, sustained or delayed release of the active ingredient after administration to the patient. The compositions are preferably formulated in a unit dosage form, each dosage containing from about 5 to about 500 mg, more usually about 25 to about 300 mg, of the active ingredient. However, it will be understood that the therapeutic dosage administered will be determined by the physician in the light of the relevant circumstances including the condition to be treated, the choice of compound to be administered and the chosen route of administration, and therefore the above dosage ranges are not intended to limit the scope of the invention in any way. The term "unit dosage form" refers to physically discrete units suitable as unitary dosages for human subjects and other mammals, each unit containing a predetermined quantity of active material calculated to produce the desired therapeutic effect, in association with a suitable pharmaceutical carrier.

In addition to the above formulations, the compounds of the present invention may be administered topically. Topical formulations are ointments, creams, and gels.

Ointments generally are prepared using either (1) an oleaginous base, i.e., one consisting of fixed oils or hydrocarbons, such as white petrolatum or mineral oil, or (2) an absorbent base, i.e., one consisting of an anhydrous substance or substances that can absorb water, for example anhydrous lanolin. Customarily, following formation of the base, whether oleaginous or absorbent, the active ingredient (compound) is added to an amount affording the desired concentration.

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Creams are oil/water emulsions. They consist of an oil phase (internal phase), comprising typically fixed oils, hydrocarbons, and the like, such as waxes, petrolatum, mineral oil, and the like, and an aqueous phase (continuous phase), comprising water and any water-soluble substances, such as added salts. The two phases are stabilized by use of an emulsifying agent, for example, a surface active agent, such as sodium lauryl sulfate; hydrophilic colloids, such as acacia colloidal clays, veegum, and the like. Upon formation of the emulsion, the active ingredient (compound) customarily is added to an amount to achieve the desired concentration.

Gels comprise a base selection from an oleaginous base, water, or an emulsion-suspension base, such as aforedescribed. To the base is added a gelling agent that forms a matrix in the base increasing its viscosity. Examples of gelling agents are hydroxypropyl cellulose, acrylic acid polymers, and the like. Customarily, the active ingredient (compounds) is added to the formulation at the desired concentration at a point preceding addition of the gelling agent.

The amount of compound incorporated into a topical formulation of invention is not critical; the concentration should only be a range sufficient to permit ready application of the formulation to the an affected tissue area in an amount that will deliver the desired amount of compound. The customary amount of topical formulation to be applied to an affected tissue will depend upon an affected tissue size and concentration of compound in the formulation. Generally, the formulation will be applied to the an affected tissue in an amount affording from about 1 to about 500 μg compound per cm^2 of an affected tissue. Preferably, the applied amount of compound will range from about 30 to about 300 $\mu\text{g}/\text{cm}^2$, more preferably, from about 50 to about 200 $\mu\text{g}/\text{cm}^2$, and, most preferably, from about 60 to about 100 $\mu\text{g}/\text{cm}^2$.

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The following formulation examples are illustrative only and are not intended to limit the scope of the invention in any way.

5

Formulation 1

Hard gelatin capsules are prepared using the following ingredients:

	Quantity (mg/capsule)
Active ingredient	250
starch, dried	200
magnesium stearate	10
	<hr/>
Total	460 mg

The above ingredients are mixed and filled into hard gelatin capsules in 460 mg quantities.

10

Formulation 2

A tablet is prepared using the ingredients below:

15

	Quantity (mg/capsule)
Active ingredient	250
cellulose, microcrystalline	400
silicon dioxide, fumed	10
stearic acid	5
	<hr/>
Total	665 mg

The components are blended and compressed to form tablets each weighing 665 mg.

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Formulation 3

An aerosol solution is prepared containing the following components:

	Quantity (mg/capsule)
Active ingredient	0.25
ethanol	29.75
Propellant 22 (chlorodifluoromethane)	<u>70.00</u>
Total	100.00

- 5 The active compound is mixed with ethanol. The mixture is added to a portion of the Propellant 22, cooled to -30°C and transferred to a filling device. The required amount is then fed to a stainless steel container and diluted with the remainder of the propellant. The valve units are then fitted
- 10 to the container.

Formulation 4

- 15 Tablets each containing 60 mg of active ingredient are made as follows:

	Quantity (mg/capsule)
Active ingredient	60 mg
starch	45 mg
microcrystalline cellulose	35 mg
polyvinylpyrrolidone (as 10% solution in water)	4 mg
sodium carboxymethyl starch	4.5 mg
magnesium stearate	0.5 mg
talc	<u>1 mg</u>
Total	150 mg

The active ingredient, starch and cellulose are passed through a No. 45 mesh U.S. sieve and mixed thoroughly. The solution of polyvinylpyrrolidone is mixed with the resultant

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powders which are then passed through a No. 14 mesh U.S. sieve. The granules so produced are dried at 50°C and passed through a No. 18 mesh U.S. sieve. The sodium carboxymethyl starch, magnesium stearate and talc, previously passed
5 through a No. 60 mesh U.S. sieve, are then added to the granules which, after mixing, are compressed on a tablet machine to yield tablets each weighing 150 mg.

10

Formulation 5

Capsules each containing 80 mg of medicament are made as follows:

	Quantity (mg/capsule)
Active ingredient	80 mg
starch	59 mg
microcrystalline cellulose	59 mg
magnesium stearate	2 mg
Total	200 mg

The active ingredient, cellulose, starch and magnesium
15 stearate are blended, passed through a No. 45 mesh U.S. sieve, and filled into hard gelatin capsules in 200 mg quantities.

Formulation 6

20 Suppositories each containing 225 mg of active ingredient may be made as follows:

	Quantity (mg/capsule)
Active ingredient	225 mg
saturated fatty acid glycerides	2,000 mg
Total	2,225 mg

The active ingredient is passed through a No. 60 mesh U.S. sieve and suspended in the saturated fatty acid glycerides
25 previously melted using the minimum heat necessary. The

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mixture is then poured into a suppository mold of nominal 2 g capacity and allowed to cool.

5

Formulation 7

Suspensions each containing 50 mg of medicament per 5 mL dose are made as follows:

	Quantity (mg/capsule)
Active ingredient	50 mg
sodium carboxymethyl cellulose	50 mg
syrup	1.25 mL
benzoic acid solution	0.10 mL
flavor	q.v.
color	q.v.
purified water to total	5 mL

10 The medicament is passed through a No. 45 mesh U.S. sieve and mixed with the sodium carboxymethyl cellulose and syrup to form a smooth paste. The benzoic acid solution, flavor and color are diluted with some of the water and added, with stirring. Sufficient water is then added to produce the required volume.

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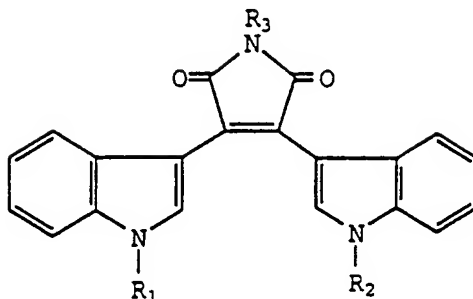
Formulation 8

An intravenous formulation may be prepared as follows:

	Quantity (mg/capsule)
Active ingredient	250 mg
isotonic saline	1000 mg
20 The solution of the above ingredients is administered intravenously at a rate of 1 mL per minute to a subject in need of treatment.	

We claim:

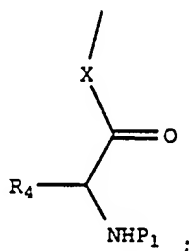
1. A compound of the formula:



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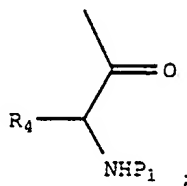
wherein:

R₁ is of the formula:



10

R₂ is hydrogen, alkyl, acyl, alkoxyalkyl, hydroxyalkyl, aminoalkyl, monoalkylaminoalkyl, dialkylaminoalkyl, acylaminoalkyl, N₃-alkyl, or an amino acid of the formula:



15

R₃ is H or CH₃;

R₄ is independently an amino acid side chain;

20

X is -(CH₂)_n-NH-, -(CH₂)_n-O-, phenylene-NH-, phenylene-O-, or a bond;

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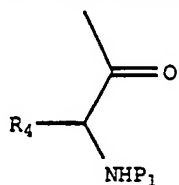
P_1 is independently H, alkyl, or an amino protecting group; and

n is independently 1, 2 or 3;

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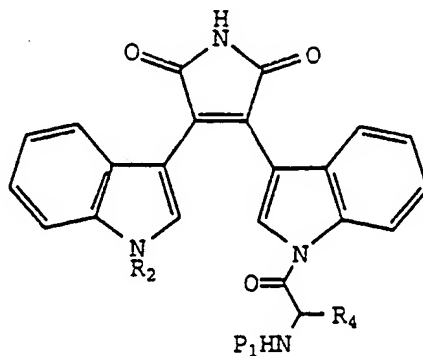
or a pharmaceutically acceptable salt or solvate thereof.

2. A compound of Claim 1, wherein R_2 is C5-C7 alkyl, acyl, alkoxyalkyl, hydroxyalkyl, aminoalkyl, monoalkylaminoalkyl, dialkylaminoalkyl, acylaminoalkyl, N3-alkyl, or an amino acid of the formula:



15

3. A compound of Claim 2 of the formula:



wherein:

- R_2 is aminoalkyl, monoalkylaminoalkyl, dialkylaminoalkyl;

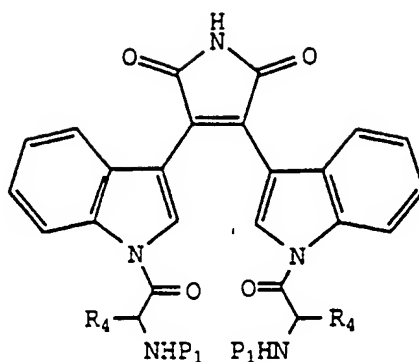
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R_4 is H, CH_3 , , or ; and
 P_1 is H, t-butoxycarbonyl, or benzyloxycarbonyl.

25

4. A compound of Claim 3 of the formula:

- 32 -

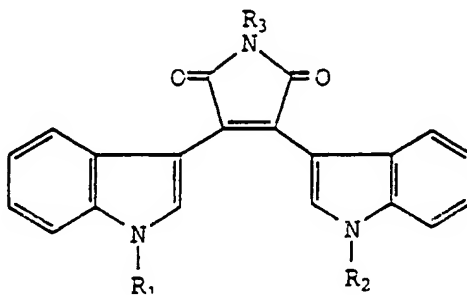


wherein:

R₄ is independently an amino acid side chain; and

5 P₁ is independently H, t-butoxycarbonyl, or benzyloxycarbonyl.

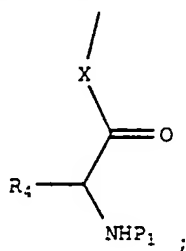
5. A compound of the formula:



10

wherein:

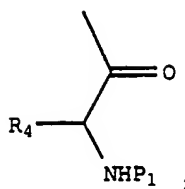
R₁ is of the formula:



15

R₂ is hydrogen, alkyl, acyl, alkoxyalkyl, hydroxyalkyl, aminoalkyl, monoalkylaminoalkyl, dialkylaminoalkyl, acylaminoalkyl, N₃-alkyl, or an amino acid of the formula:

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R₃ is H or CH₃;

5 R₄ is an amino acid side chain;

X is -(CH₂)_n-NH-, -(CH₂)_n-O-, phenylene-NH-,
phenylene-O-, or a bond;

10 P₁ is H, alkyl, or an amino protecting group; and

n is 1, 2 or 3;

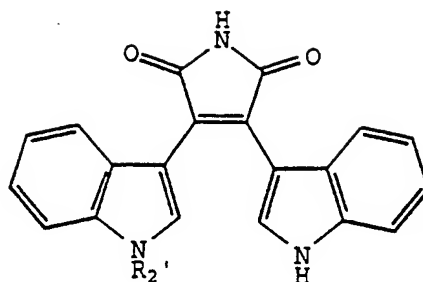
or a pharmaceutically acceptable salt or solvate
15 thereof.

6. A compound as claimed in any of claims 1 to 5
for use as a pharmaceutical.

20 7. A pharmaceutical formulation comprising as an
active ingredient a compound as claimed in any one of claims
1 to 5, associated with one or more pharmaceutically
acceptable excipients, carriers, or diluents therefor.

25 8. A process for preparing a compound as claimed
in any one of claims 1 to 5, which comprises: Reacting a
compound of the formula:

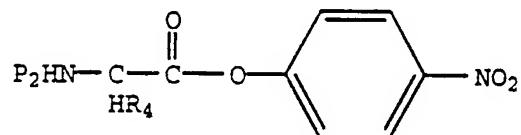
- 34 -



wherein:

R_2' is hydrogen, alkyl, acyl, alkoxyalkyl, hydroxyalkyl, aminoalkyl, monoalkylaminoalkyl, dialkylaminoalkyl, acylaminoalkyl, or N_3 -alkyl;

with an activated amino acid of the formula:



10

wherein P_2 is an amino protecting group; and R_4 is an amino acid side chain.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/07791

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) : Please See Extra Sheet.

US CL : 548/455 ; 514/414

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 548/455 ; 514/414

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAS ONLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 5,380,746 (BARTH ET AL) 10 January 1995, columns 1-10, and column 109, embracing claim 1.	1-8
Y	DE, A, 39 14 764 (GODECKE AG) 08 November 1990, See Abstract.	1-8

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:	*T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
A document defining the general state of the art which is not considered to be part of particular relevance	*X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
E earlier document published on or after the international filing date	*Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	*G* document member of the same patent family
O document referring to an oral disclosure, use, exhibition or other means	
P document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search

21 SEPTEMBER 1995

Date of mailing of the international search report

13 OCT 1995

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231

Facsimile No. (703) 305-3230

Authorized officer

DAVID B. SPRINGER

Telephone No. (703) 308-1235

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/07791

A. CLASSIFICATION OF SUBJECT MATTER: IPC (6):

C07D 403/14; A61K 31/40